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RESEARCH ARTICLE

IS TAUTOMERIZATION RELEVANT TO THE EXISTENCE OF MISPAIRED CYTOSINE-GUANINE (C-G)?

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ABSTRACT

The pairing of guanine(G) and cytosine(C) in a different manner from the Watson Crick(WC) type CG have been investigated by using DFT (B3LYP/6-31+G(d,p)) calculations. Certain sites of G and C in WC GC are very sensitive to proton that may result destabilization of hydrogen bonds . Several tautomers may be formed by destabilizing WC CG due to the effect of proton. Subsequently paring of tautomers through H-bonding in a different manner might lead to mispaired CG. The possibility of transforming WC CG directly to mispaired CG has been explored from the potential energy scan, but we have found large activation energies ranging from 40.946 kcal/mol to 71.347 kcal/mol in most cases except for CG-P7 and CG-P3. Hence, the tautomerization reaction might be the feasible pathway for generating mispaired CG.

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INTRODUCTION

The guanine (G) and cytosine (C) nucleobases can exist in various tautomeric forms and it is a very important process related to the emergence of several diseases and the change of genetic code (Blas *et al*., 2004; Barsky and Colvin, 2000; Rueda *et al*., 2001; Raczyńska *et al*., 2013; Perez *et al*., 2010; Tomic *et al*., 2005; Wang *et al*., 2011; Faustino *et al*., 2009; Podolyan *et al*., 2003; Vonderach *et al*., 2012). Among the four nucleobases, guanine (G) is more susceptible to tautomerization and some tautomers of G are shown in Figure I. It may be noted that once the guanine is generated in WC CG, the subsequent generation of cytosine tautomer is possible . Some cytosine tautomers are shown in Figure I. It is likely that the tautomers of G and C may pair up in a different way through H-bonding to form mispaired CG**.** The rearrangement of H-atom present at certain groups in guanine and cytosine are likely to occur during tautomerization of these nucleobases. Several base pair combinations may be formed from these tautomers through hydrogen bonding. Prototropic rearrangement is one of the tautomerization mechanisms for generating G and C tautomers, since intramolecular proton transfer in WC CG and also in hydrated guanine has been discussed in many literatures (Podolyan *et al*., 2003; Vonderach *et al*., 2012). Some tautomers might not be so stable under normal condition and they may combine to generate several base pairs other than WC CG base pair. These G and C tautomers may interact to form mispaired CG through H-bonding in a different manner. However, no substantial information is found on the pairing of tautomers. The pairing of nucleobase tautomers usually depends on the H-bonding capacity of the counter nucleobases G and C. Most of the available mismatched CG may also be associated with the mechanism of prototropic transformation among tautomers due to the unambiguous small differences on the stability of these tautomers (Nurbosyn *et al*., 1998; Guerra *et al*., 2006; Michelson *et al*., 2012; Seela *et al*., 2005; Pluharova *et al*., 2011; Ho *et al*., 2011; David, 2013; Sponer *et al*., 1999). Hydrogen bonds between WC GC may be affected by the surrounding water molecules and ions that may be relevant to tautomerization reactions (Pluharova *et al*., 2011; Ho *et al*., 2011). The H-bonding between various sites of G and C other than WC type H-bonding are observed in several sequences of DNA. It may be due to the change of acidic nature of H-bonding sites in WC CG after interaction with surrounding ions. As we know that the acid-base characteristics of various donor-acceptor sites of G, and also for C are not very different. The mechanism of H-migration within certain sites is usually easy for nucleobases having narrow difference of basicities. Subsequently, it is possible that nucleobase tautomers may

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combine to form several mispaired CG other than WC CG, which leads to distortion of normal DNA structure. This aspect is very important in DNA mutation as well as in the evolution of chronic diseases. Initially, it is essential to know the circumstances under which WC CG get destabilized, and one can further explore the pairing of complementary tautomers to form mispaired CG. Hence, the present study has been taken up to examine several strategies of forming tautomer pairs to generate mispaired CG.

COMPUTATIONAL METHODS

Complete geometry optimization of tautomers and mispaired CG have been carried out by using B3LYP/6-31+G(d,p) calculations. The interaction energies ΔE, changes of thermal energies (ΔH) and Gibbs free energies (ΔG) are estimated for these mispaired CG. The BSSE are also calculated to estimate the error in the interaction energies of these H-bonded tautomer pairs due to insufficient basis set. The Gibb's free energies are calculated at 298K. To understand the destabilization of WC CG due to interaction of proton at the basic sites present outside hydrogen bonded region of WC CG, we have computed destabilization energies of protonated WCGC with B3LYP/6-31+G (d,p) calculations. We have also examined the reaction pathway for the conversion of WC CG to mispaired CG. Before performing potential energy scan, the hypothetical transition state structures are carefully identified. All calculations were carried out with Gaussian 03 program code (Trucks *et al*., 2003). It is important to estimate the equilibrium constants (K_E) and the pK_E of mispaired CG with respect to WC CG. The feasibility of conversion from WC CG to mispaired CG may be analysed from these computed values. The pK_E and K_E values are calculated from the following equation.

$$pK_E = \frac{\triangle G}{2.303\ RT}$$

Where, K_E = Equilibrium constant, ΔG = Gibb's free energy change, T = 298K, and R = Gas constant.

$$K_E = e^{-\Delta G/2.303RT}$$

The computed values may be taken to understand the equilibration of WC GC and mispaired GC.

RESULTS AND DISCUSSION

Typical guanine tautomers, such as G2, *cis*G1 and *trans*G3, and cytosine tautomers *trans* C1, *cis*C2 and *cis*C1 are some of the stable tautomers of G and C (Table I). These tautomers may be formed due to slight change of solution pH, considering that protonation/deprotonation reaction is the relevant pathway of tautomerization and the protonation energies of several sites of G and C are not very different (Table II). It is possible that less stable tautomers may also be formed other than major tautomers. Some of the basic sites of WC CG are sensitive to proton or water molecules that may destabilize the hydrogen bonding in WC CG. Considering that G is the most basic nucleobase we have computed the protonation energies and interaction energies after protonation. Table III indicates significant changes in these values. There are possibilities of forming anionic tautomers unlike the formation of keto/enol tautomerization. Here, the study has been taken up mainly for the tautomerization due to H- migration mechanisms. The tautomers at the same time may generate several mispaired CG (Table IV). Figure 2 provides the structural features of mispaired CG, which are formed through hydrogen bonding between two compatible sites of C and G tautomers. The intermolecular H-bonding patterns in these mispaired CG are different from WC CG, and the H-bonding patterns and the H-bond distances are shown in Figure II and Table V. In these structures the H-bonds may be either twisted or planar depending on the H-bonding capacity of N1, N2 and O6 of G with the sites O2, N3 and N4 of C. Comparison of the H-bond lengths and angles of these mispaired GC are useful to the understanding of stable mispaired CG. The ΔE, ΔG and ΔH values of mispaired CG are shown in Table VI. Figure III illustrates the relative change of the electronic energies with respect to WC CG.

The H-bond patterns and their characteristics may be used to extract information about the more responsible H-bond in mispaired CG pairs. In several of these mispaired CG, the H-bond strengths are not equal, which indirectly related to the differences of the H-bond length. The H-bonding patterns in WC CG may be compared with other mispaired CG, herein the middle bond Hm is 1.902 Å, and the upper(Hu) and lower (H_l) bonds are slightly shorter i.e 1.750 Å and 1.895 Å respectively(Table V). It is however possible that the Hu and H_l bond strengths are stronger than the Hm. If the interaction between tautomers G and C through these H-bonds are concerned, the other two H-bonds usually contribute more in the pairing than Hm bond. Pairing of C and G reflect on the characteristic of H-bonding abilities of H_l, Hm and Hu. The H-bonds in CG-P3 are less than 2 Å, the formation of closer H-bond at the upper region (Hu) than Hm and H_l has been indicated. Hence, it is likely that Hm exclusively stabilize pairing of tautomers in CG-P3. As seen in CG-P3, two H-bonds are involved in tautomers C and G pairing. The stability of mispaired GC is thermodynamical domain, but still the H-bonding pattern and H-bond distances may be useful to understand H-bond strengths. Similarly, there are other structures, CG-P5 and CG-P7, where H_l is much longer than H_u and H_m in their structures. From these observations, the change of overall sugar conformation attached at the nucleotide of DNA may be indirectly guessed. We also observed equal H-bond lengths in CG-G6, with slightly longer than those of other mispaired CG. These results show that some of the donor sites may act as driving site for H-bond formation in mispaired CG.

It is generally assumed that guanine is the most basic nucleobase. So tautomerization of this nucleobase may be predominant compared to C under certain conditions. The results obtained from quantum mechanical calculations can be used to estimate the comparative stability of several tautomeric structures (Figure VI). It is also possible that unstable tautomers may instantaneously pair up to form several stable mispaired GC. Hence, the extent of acidic/basic characters of these sites can determine the interaction ability of tautomers. For the nispaired CG shown in Figure II, the corresponding zero point energy, ΔH and ΔG are also evaluated (Tables VI).

Table I. Computed ΔE, ΔH, ΔG, K_{eq} and ZPE of different nucleobase tautomers with B3LYP/6-31+G(d,p) methods of calculations

Nucleobase	Tautomers	Energies (kcal/mol)	K_{eq}	ZPE (kcal/mol)
G→	G2	19.477[a], 19.522[b], 19.288[c]	$2.5x10^{-15}$	-0.423
	*1cis*G4	21.628[a], 21.610[b], 22.083[c]	$7.9x10^{-17}$	-0.078
	*cis*G1	0.720[a], 0.336[b], 1.043[c]	0.172	-0.392
	*cis*G5	22.232[a], 22.255[b], 22.828[c]	$1.9x10^{-17}$	-0.232
	*1trans*G5	23.844[a], 23.956[b], 24.411[c]	$1.3x10^{-18}$	-0.299
	*cis*G4	36.127[a], 36.234[b], 36.176[c]	$3.2x10^{-27}$	-0.227
	*trans*G3	15.985[a], 15.492[b], 15.494[c]	$4.5x10^{-12}$	-0.496
C→	*trans*C1	2.472[a], 2.960[b], 2.504[c]	0.015	-0.902
	*cis*C2	19.929[a], 34.159[b], 34.377[c]	$6.4x10^{-16}$	-0.304
	*cis*C1	1.805[a], 4.627[b], 4.203[c]	$8.4x10^{-4}$	0.790
	*1trans*C2	23.433[a], 24.202[b], 23.447[c]	6.8x10-18	0.481
	*1cis*C2	34.606[a], 34.159[b], 34.377[c]	$6.7x10^{-26}$	-0.303

a→ ΔE, electronic energy change for the reaction, b→ ΔH, Enthalpy change for the reaction, c→ ΔG, Free energy change for the reaction, Keq→ Equillibrium constant of the reaction, ZPE→ zero point energy. *Numbering indicate the position of proton involved in tautomerization

Table II. Variations of protonated energies of different nucleobase tautomers with B3LYP/6-31+G(d,p)

Position of proton in nucleobases	Tautomers	Protonation energies (kcal/mol)
G7	1*cis*G4	-268.384
G6	*trans*G1	-241.156
G3	1*trans*G4	-255.978
G2	G2	-224.319
G1	*trans*G4	-237.920
C1	1*cis*C2	-240.334
C2	1*trans*C2	-268.978
C3	1*trans*C2	-269.531

*Numbering indicate the position of proton involved in tautomerization

Table III. Interaction energies and protonation energies of protonated GC pairs calculated using B3LYP/6-31+G(d,p)

Position of proton in base pairs	Interaction energies (IE = PBP – B - BH^+) (kcal/mol)	Protonation energies (PE = PBP - BP) (kcal/mol)
G1	-73.574	-257.495
G3	-50.306	-249.377
G7	-41.073	-247.585

IE→ Interaction Energies, B→ Nucleobase, BH^+→ Protonated Base, PBP→ Protonated Base Pairs, BP→ Base Pairs, PE→ Protonation energies
*Numbering indicate the position of proton.

Table IV. Computed interaction energies of normal base pair and tautomerized base pairs and BSSE energies with B3LYP/6-31+G(d,p)

Base pair	Interaction Energies, IE = E_{BP} - E_B - E_{BH}+(kcal/mol)	BSSE Energies (kcal/mol)
	B3LYP/6-31+G(d,p)	B3LYP/6-31+G(d,p)
CG → -26.017		
Tautomers → 1. CG-P1	-17.396	0.793
2. CG-P2	-48.935	0.669
3. CG-P3	-19.065	1.092
4. CG-P4	-49.720	0.891
5. CG-P5	-16.792	0.847
6. CG-P6	-8.798	0.725
7. CG-P7	-26.664	1.548
8. CG-P8	-16.620	1.086

E_{BP} → Energies of base pairs or tautomerised base pairs
E_B → Energies of tautomerised base Guanine(G)
E_{BH}+→ Energies of tautomerised base Cytosine(C)
BSSE→ Basis set superposition error.
*Numbering indicate position of proton in CG.

Table V. Computed H-bond distances in CG and tautomerized pairs

Base pairs	Tautomerised WC base pairs	H-bond distance (Å)	Planarity
CG	-	H_u →1.750	
		H_m→1.902	Planar
		H_l →1.895	
CG-P1		H_u →2.138	
		H_m→1.973	Planar
		H_l →1.793	
CG-P2		H_u →2.246	
		H_m→2.062	Planar
		H_l →1.867	
CG-P3		H_u →1.683	
		H_m→1.837	Planar
		H_l →1.975	
CG-P4		H_u →2.003	Twisted(35.24^0)
		H_l →1.907	
CG-P5		H_u →1.632	
		H_m→1.873	Planar
		H_l →2.182	
CG-P6		H_u →2.261	
		H_m→2.104	Planar
		H_l →2.058	
CG-P7		H_u →1.678	
		H_m→2.166	Planar
		H_l →2.464	
CG-P8		H_u →2.222	
		H_m→1.889	Planar
		H_l →1.609	

The value in parenthesis ( ) indicates torsional angle.

Table VI. Computed ΔE, ΔH, K_E, ΔG and ZPE of different tautomerized base pairs with B3LYP/6-31+G(d,p) calculations.

WC	Mispaired CG	Energies of the reactions (kcal/mol)	K_E	pK_E
	CG-P1			
	CG-P2	31.585[a], 32.997[b], 30.613[c], -0.418[d]	$3.8x10^{-23}$	22.415
	CG-P3	18.849[a], 19.447[b], 16.938[c], -0.326[d]	$4.0x10^{-23}$	12.402
	CG-P4	22.849[a], 23.421[b], 22.937[c],-0.050[d]	$1.6x10^{-17}$	16.795
CG	CG-P5	15.072[a], 23.023[b], 19.945[c], 0.009[d]	$2.5x10^{-15}$	14.604
	CG-P6	33.737[a], 32.997[b], 32.558[c], 0.489[d]	$1.4x10^{-24}$	23.839
	CG-P7	44.759[a], 46.171[b], 44.979[c],-0.407[d]	$1.2x10^{-23}$	32.934
	CG-P8	70.269[a], 70.887[b], 69.319[c],-1.450[d]	$1.8x10^{-51}$	50.756
		44.844[a], 44.791[b], 42.407[c],-0.624[d]	$1.0x10^{-31}$	30.986

a→ ΔE, Electronic energy change for the reaction, b→ ΔH, Enthalpy change for the reaction, c→ ΔG, Free energy change for the reaction, d→ ΔZPE, Zero point energy, K_E→ Equilibrium constants of the reaction. * Numbering indicate the position of proton.

Table VII. Variations of activation energies during conversion of normal base to its tautomerised base

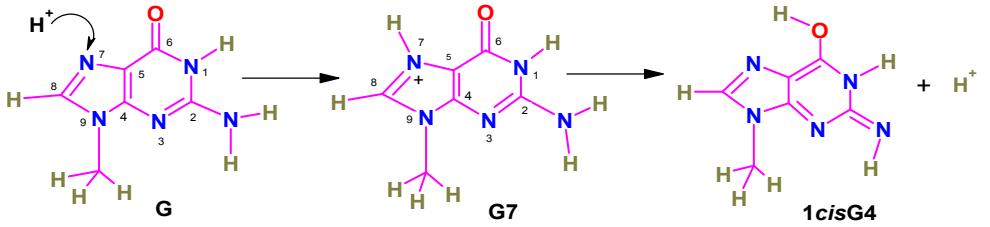
Conversions	ΔA (kcal/mol)
	B3LYP/6-31+G(d,p)
G1→*trans*G4	6.445
G2→G2 tautomer	63.897
G3→1*trans*G4	18.819
G6→*trans*G1	16.386
G7→1*cis*G4	5.123
C1→1*cis*C2	28.245
C2→1*trans*C2	9.976
C3→1*trans*C2	35.420

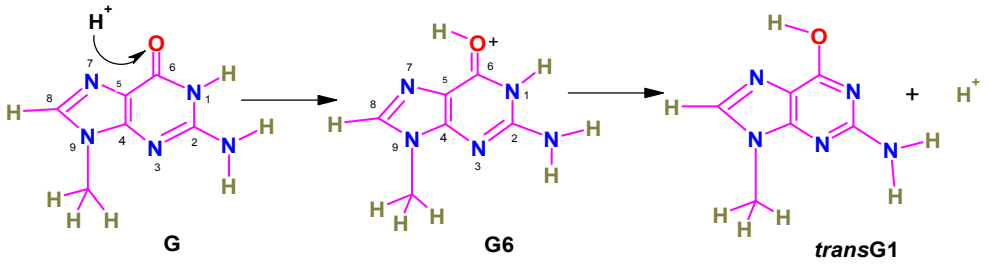
*numbering indicates the position of proton

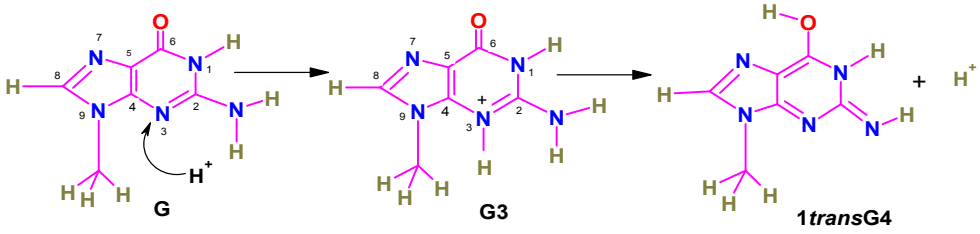
Table VIII. Variation of activation energies during conversion of WCGC to mispaired GC

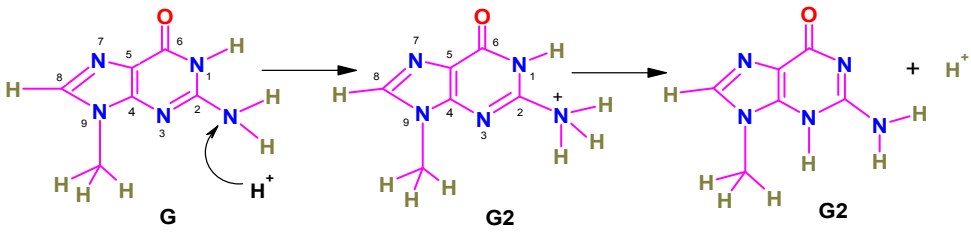
Base pair WC	Mispaired CG	ΔA (kcal/mol)
		B3LYP/6-31+G(d,p)
CG	CG-P1	41.598
	CG-P2	59.123
	CG-P3	15.818
	CG-P4	71.347
	CG-P5	40.946
	CG -P6	49.010
	CG-P7	3.990
	CG-P8	51.823

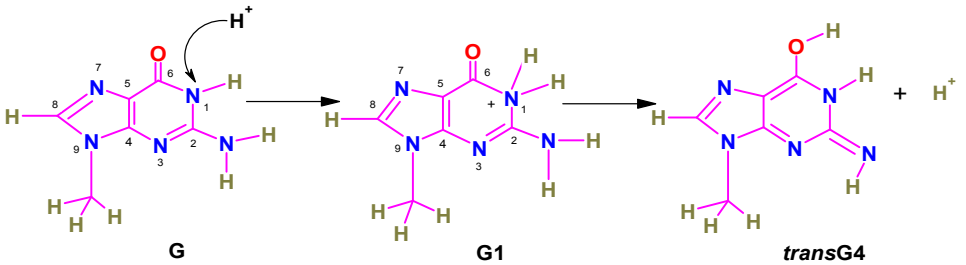
* Numbering indicate the position of proton











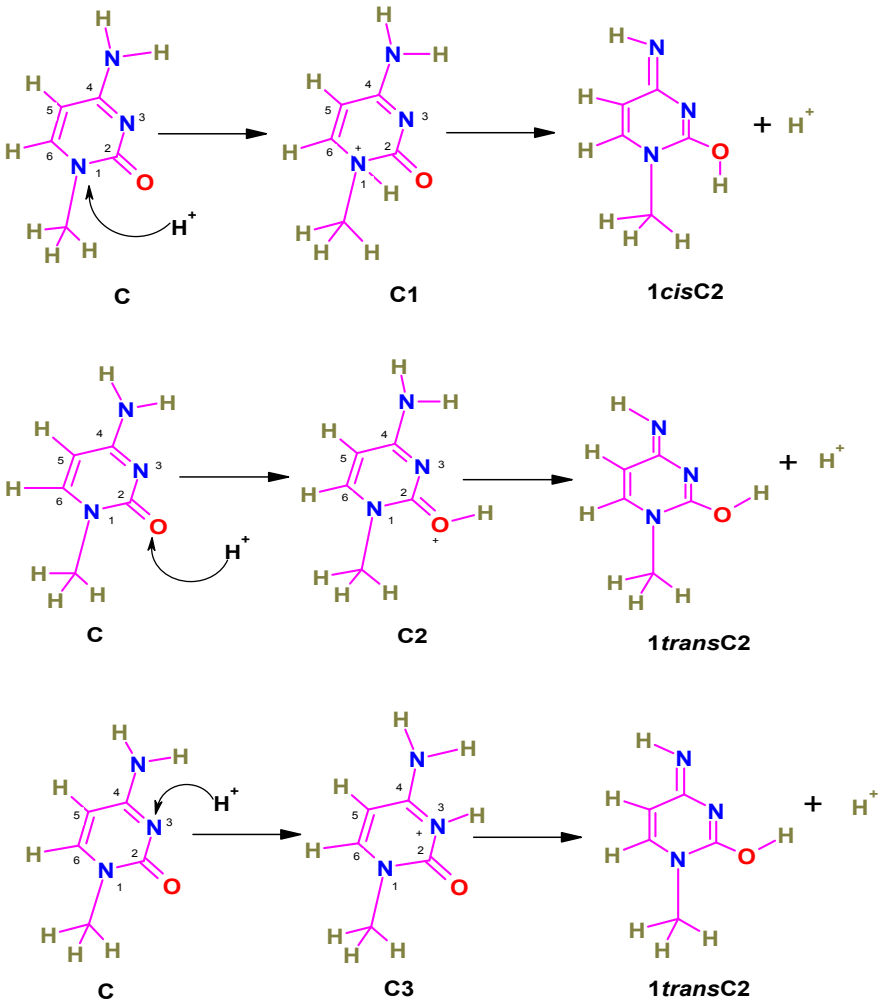
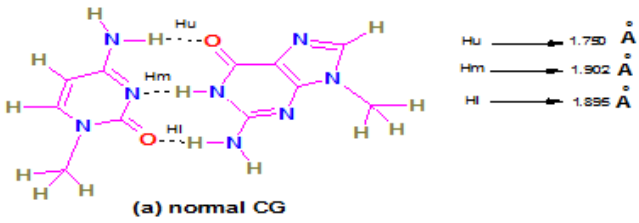
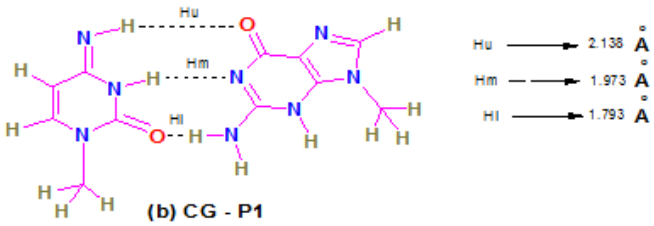
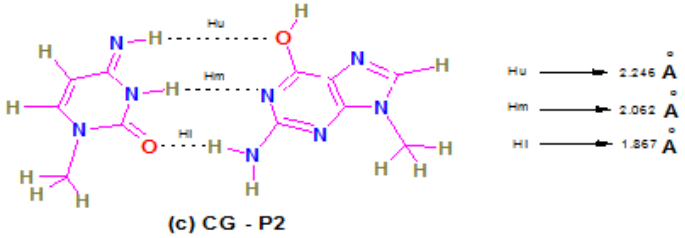


Figure 1. Conversion of normal nucleobase to tautomers through protonated forms







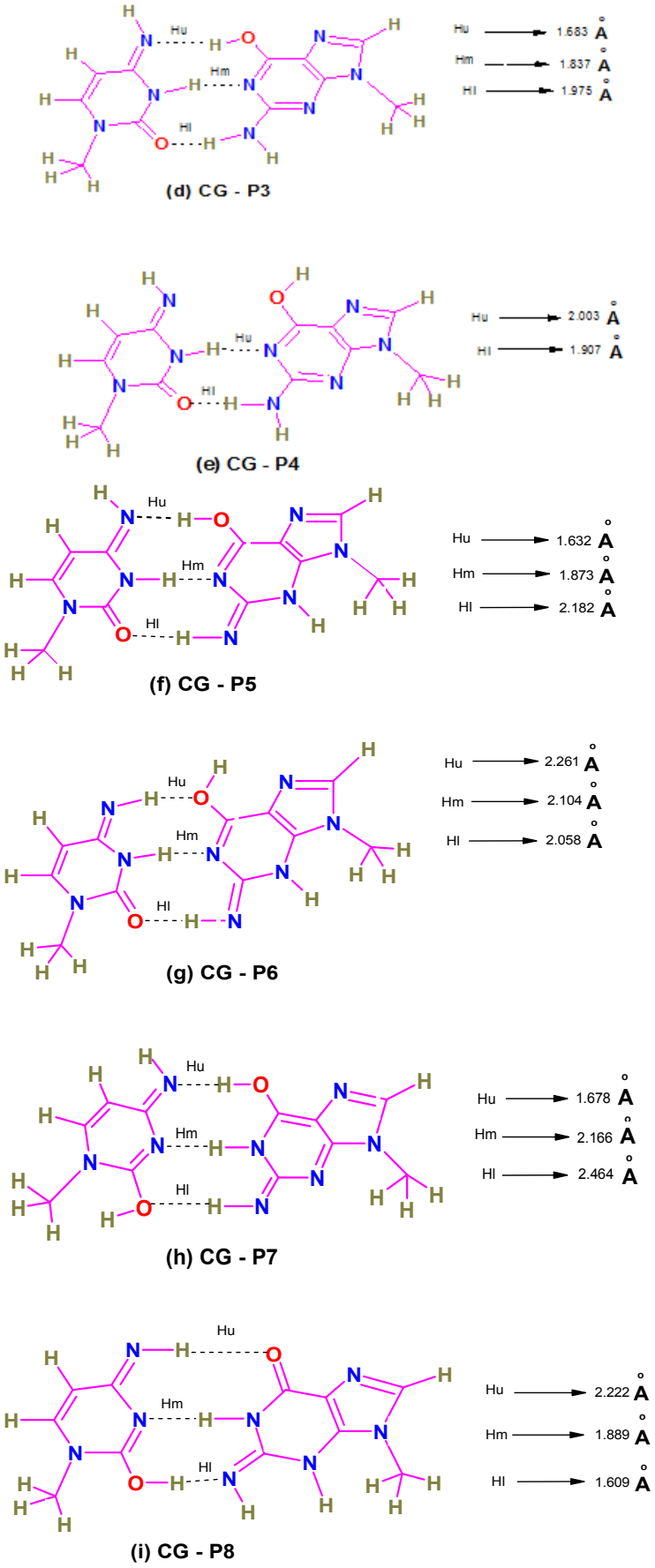


Figure 2. Structures of tautomerized CG base pairs (a) normal GC (b) CG-P1(c) CG-P2 (d) CG-P3 (e) CG-P4 (f) CG-P5 (g) CG-P6 (h) CG-P7 (i) CG-P8

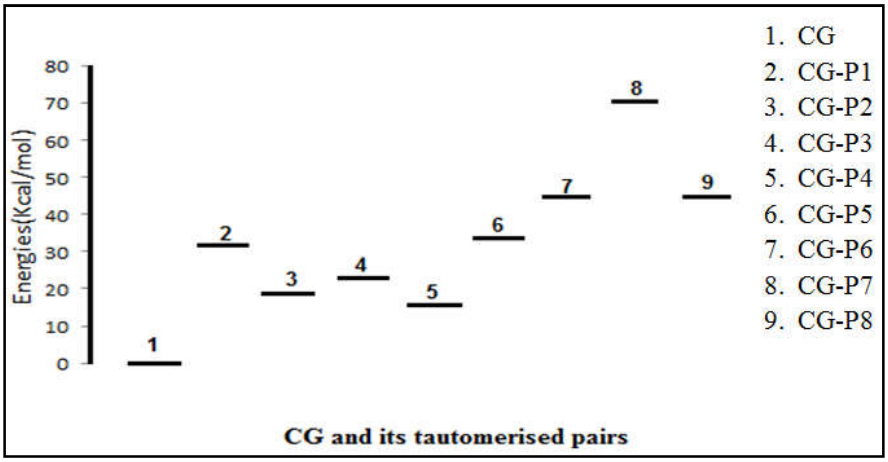


Figure 3. Variation of energies of tautomerized base pairs with respect to normal CG

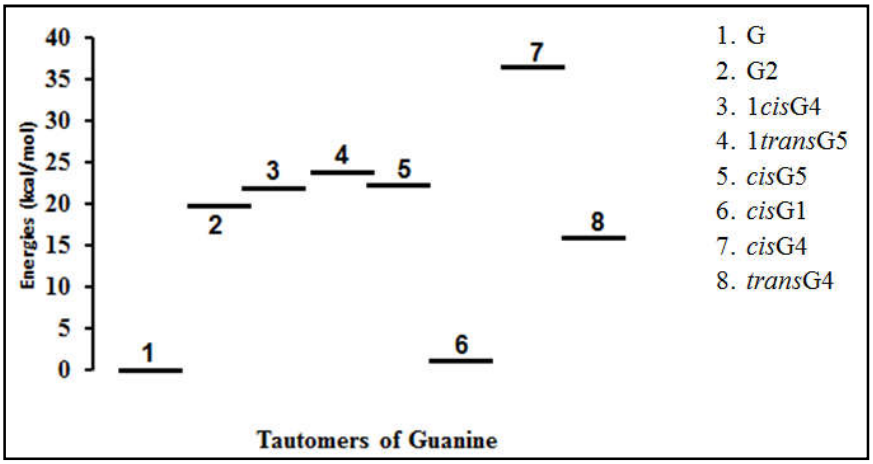
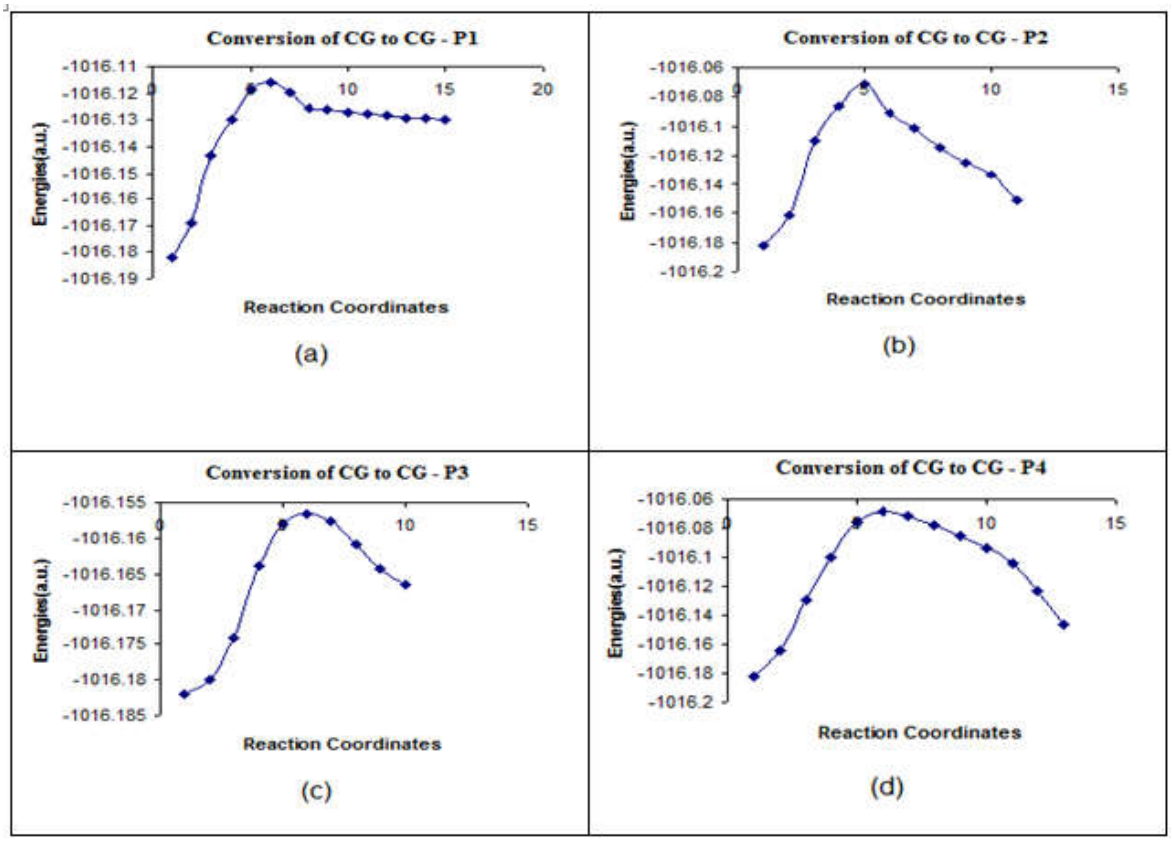


Figure 4. Variation of energies of guanine tautomers with respect to normal G



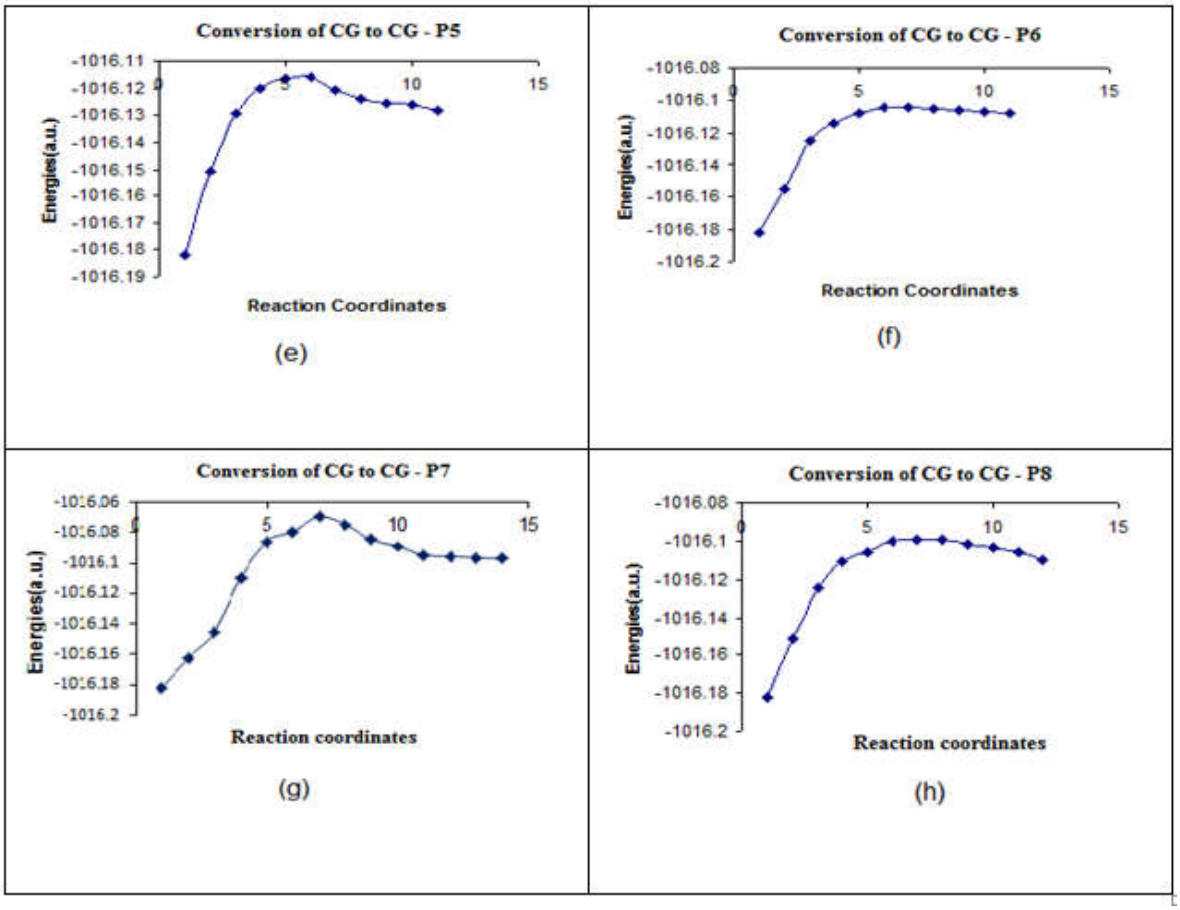
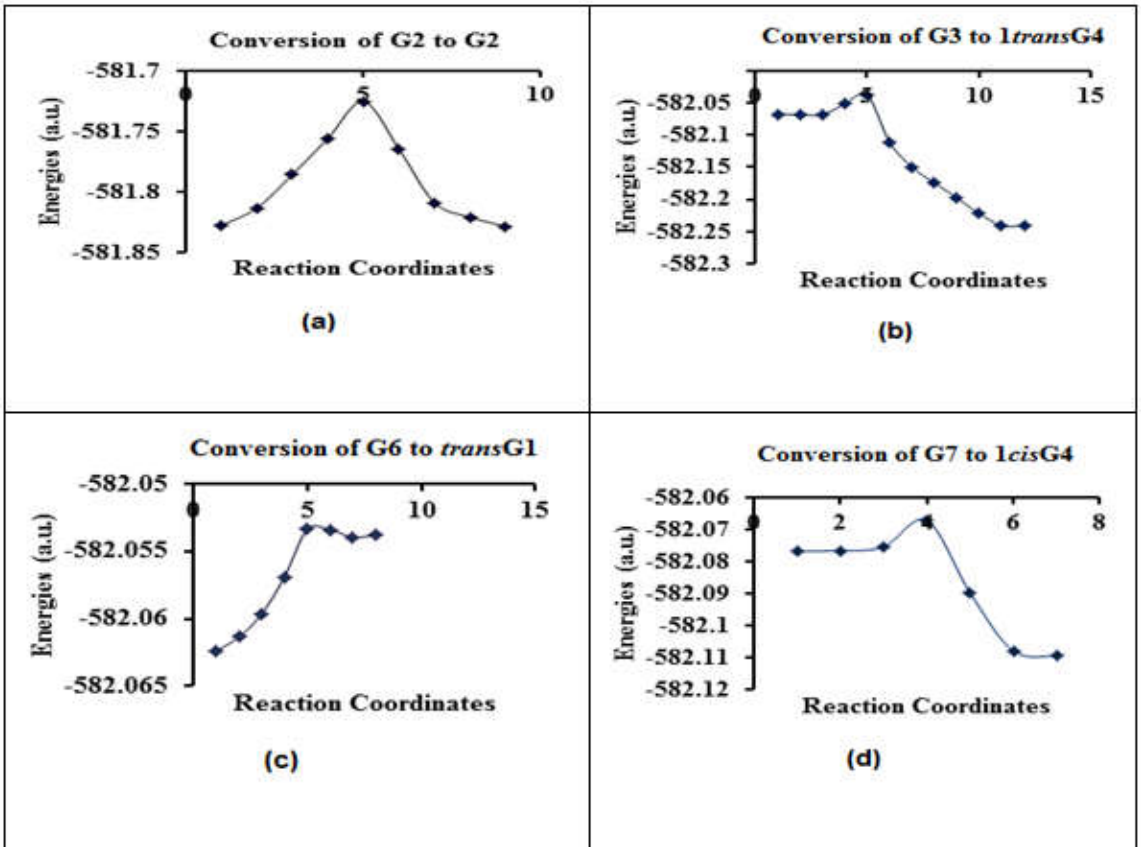


Figure 5. Potential energy plot for conversion of
(a) CG→ (CG-P1) (b) CG→ (CG-P2) (c) CG→ (CG-P3) (d) CG→ (CG-P4)
(e) CG→ (CG-P5) (f) CG→ (CG-P6) (g) CG→ (CG-P7) (h) CG→ (CG-P8)



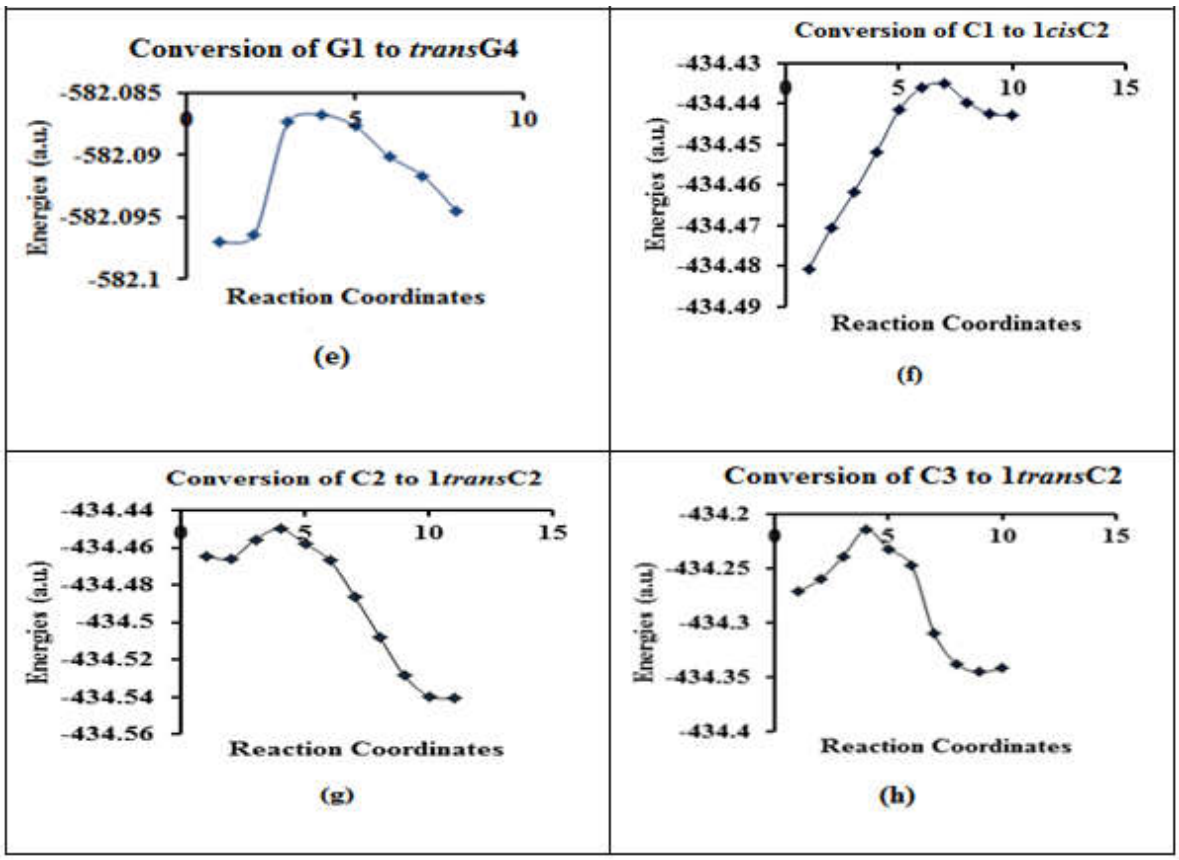


Figure 6. Potential energy plots for conversion of nucleobases through protonated intermediates
(a) G2→ G2 (b) G3→ 1*trans*G4 (c) G6→ *trans*G1 (d) G7→ 1*cis*G4
(e) G1→ *trans*G4 (f) C1→ 1*cis*C2 (g) C2→ 1*trans*C2 (h) C3→ 1*trans*C2

Most of the structures have negative vibrational mode. However, many mispaired CG are less stable than normal CG except for CG-P2 and CG-P4. It is indeed explained the basis of H-bonding capacity among mispaired CG. For instance, the conversion of WC CG to mispaired GC may be hypothesised through some transition state. The situation for protonation/deprotonation steps to form several mispaired CG studied by choosing appropriate transition state structure feasible for the reactions. The activation energies for tautomerization through protonated intermediates are given in Table VII and the values are exceptionally high. The computed K_E and pK_E do not indicate direct transformation of WC CG to mispaired CG (Table VI). We are fully aware of the basic mechanism of reaction from WC CG to mispaired CG which is expected only after destabilization of WC CG and the possibility of tautomerization of G and C is highlighted. However, the activation energies for these reactions can be approximately estimated from the potential energy scan through some hypothetical transition state. Table VIII shows the variation of activation energies for the formation of mispaired CG pairs, and the potential energy plots shown in Figures V and VI clearly indicate endothermic reactions. Hence, in most cases tautomerization can not readily take place without additional effect on the WC CG, except for CG-P3 and CG-P7. The range of activation energies is from 3.990 kcal/mol to 71.347 kcal/mol.

From the computed values of equilibrium constants, tautomerization of G to *cis*G1 is found to be a highly favoured reaction (K_E=0.172), whereas the most feasible tautomerization is expected from C to *trans*C1(K_E= 0.015) (Table 1). The tautomerization of C to *cis*C1 is also possible compared to other tautomers of C(K_E=8.4x10^{-4}). We find wide variation of interaction energies of mispaired GC, implying a possibility of forming base pairs other than WC CG (Table IV). The GC-P4 and GC-P2 acquire larger interaction energies (-ve) than other mispaired GC. A broad various features of H-bonding in mispaired CG are observed. Note that Hu(upper), Hm(middle) and H_l(lower) H-bonds can stabilize mispaired CG, but two H-bonds (Hu and H_l) are present in some pairs. Due to these two hydrogen bonds, Hu and H_l we could observe variation of structure particularly the H-bond distances in mispaired CG pairs.

Conclusion

The rare tautomers of guanine and cytosine are less stable than their normal forms. The tautomers exist within small energy levels, so transformation of tautomers from one form to another is possible. Since the normal nucleobases are the most stable forms, the formation of rare tautomers may take place under unusual condition i.e due to the effect of proton or charged species on WC CG. Pairing of tautomer G and C is possible to form stable mispaired CG, and CG-P4 is found to be the most stable pair. The values of equilibrium constant (K_E) and pK_E indirectly demonstrate the existence of tautomers *cis*G1 and *trans* C1, whereas formation of other tautomers from directly from G and C are not indicated from the K_E values. The

existence of canonical forms of tautomers within small energy levels is a possible pathway of forming several rare tautomers. The activation energies for the transformation of WC CG to mispaired CG through hypothesized intermediates are appreciably large in most cases. Hence, it is likely that mispaired CG can be formed only from the pairing of tautomer G and C.

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